

Expression of GFP

Materials

- Autoclaved flasks, water, Schott bottles
- LB medium

Day 1: Preparations

- 20 ml LB medium with 20 µl Ampicillin is inoculated with one clone E. coli DH10b (GFP-overexpression construct being pEX_HisII backbone) and grown overnight at 37°C

Day2: Growth and Expression

- 1 L of LB medium with 1 ml Ampicillin is prepared in 5 L bottle
- for having optimal growth conditions, we aim for an OD of 0.05 to 0.1 in 1 L medium
$$\frac{0.05 \text{ or } 0.1}{OD \text{ of preculture}} \times 1000 \text{ mL} = \text{volume needed for inoculation}$$
- measure OD₆₀₀ after 90 minutes for the first time, then depending on the OD₆₀₀ every 30 minutes or every hour
- when an OD₆₀₀ of 0.6 to 0.8 is achieved take non-induced SDS-sample, centrifuge, and resuspend in 60 µl H₂O und 15-20 µl 5x SDS-buffer
- induce with IPTG to a final concentration of 1mM
- expression at 37°C overnight

Day 3: After expression

- measure OD₆₀₀ and take induced SDS-sample (as described above)
- centrifuge cell culture at 8000g, 10 min, 4°C
- store pellet at -20°C until purification